ZOOLOGICAL SCIENCE **11**: 647–662 (1994)

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REVIEW

Cell Signaling Mechanisms for Sperm Motility

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INTRODUCTION

Signal transduction mechanisms have been shown by many studies to regulate a number of cell functions in animals and plants. Binding of ligands to receptors and opening or closing of ion channels are the main triggers of these mechanisms.

Spermatozoa have long received attention due to their vigorous motility. There is too much evidence that the basic mechanochemical mechanism of flagellar motility is ATP-induced sliding of microtubules, the energy for sliding being due to ATPase activity associated with the dynein arms on the outer doublet microtubule [53]. Recent studies on sperm motility have been focused on the cell signaling mechanism for initiation of motility: completely immotile, such as teleost sperm and low motility sperm, such as mammalian sperm in the male reproductive organ, start to become motile on spawning and ejaculation. Activation of sperm motility as well as their respiration and attraction to eggs, which occurs after the initiation of motility and in response to substances released from the egg, are attractive subjects for analysis of the mechanisms underlying cell signaling. This article reviews results obtained mainly in the author and his collaborates (Chapter II and III) and other laboratories (Chapter IV) on possible regulatory mechanisms of sperm motility with two different types of trigger, changes in the external ionic or osmotic environment and ligand-receptor binding.

II INITIATION OF SPERM MOTILITY BY SIGNALS FROM THE ENVIRONMENT

Many physical and chemical factors, such as mechanical dilution, change in the environmental O_2-CO_2 tension, pH and heavy metal concentrations, have been suggested to initiate sperm motility (for review, see ref. [104]). However, Morisawa and Suzuki actually first demonstrated that external signals initiate motility of sperm of teleost fishes [107]. Their finding opened the way to analyze and clarify cell signaling systems related to motility of sperm cells.

A Domain controlling flagellar motility located in the basal region of the sperm flagellum of salmonid fishes

Spermatozoa of salmonid fishes such as rainbow trout and chum salmon are completely quiescent when the semen is diluted in the medium containing millimolar concentrations of K^+, but show motility when the semen is suspended in K^+-free medium [107, 110]. The seminal plasma of salmonid fishes contains a high concentration of K^+ and the spawning ground, fresh water of rivers has a low K^+ content [104]. Thus the experimental condition of low K^+ concentration is similar to that in natural conditions on spawning; release from the suppression of K^+ on spawning in freshwater causes the initiation of sperm motility. The effects of Na^+ and K^+ on sperm motility have also been investigated in amphidromous salmonidform species, the ayu, *Plecoglossus altivelis* [185].

Reduction of the K^+ concentration surrounding sperm on spawning in freshwater, that is, the first event triggering the initiation of sperm motility, may cause the efflux of K^+ through the K^+ channel, since blockers of the K^+ channel such as tetraethylammonium, Ba^{2+} and triphenil tin, suppress K^+ efflux and sperm motility [170, 171]. Hyperpolarization of the plasma membrane induced by K^+ efflux occurs during motility initiation of trout sperm in K^+-free medium [15, 52, 169]. A blocker of the Ca^{2+} channel, verapamil, also inhibits trout sperm motility [41, 170]. The K^+-dependent immotility of sperm can be reversed by the addition of Ca^{2+} [9, 170], implicating the occurrence of Ca^{2+} influx through the Ca^{2+} channel for initiation of motility. Okuno and Morisawa [134] showed that a low concentration of Ca^{2+} of less than $10^{-8.5}$ M is necessary for reactivation of demembranated trout sperm and that 10^{-8} M Ca^{2+} is inhibitory. Furthermore, when the cAMP concentration in the reactivating solution was changed, the concentration of Ca^{2+} for conversion of demenbranated sperm from the motile to the immotile state was not altered, suggesting that the Ca^{2+}-dependent regulating system of flagellar movement is independent of a cAMP-induced initiation mechanism. Boitano and Omoto [16] recently found that the addition of other divalent cations besides Ca^{2+} can activate trout sperm motility in the presence of K^+, suggesting that the effect of Ca^{2+} is not specific. They measured the ability of these divalent cations to hyperpolarize the membrane potential and proposed that membrane hyperpolarization rather than Ca^{2+}

Received September 19, 1994

influx is the trigger for the initiation of sperm motility. Short term intrusion of Ca^{2+} has not been detected [170], but, measurements of the fluorescence intensity of Quin-2, Fura-2 or Fluo-3 with a fluorescence spectrometer [16, 171] or by microfluorometry using a highly sensitive video camera [171] suggest the occurrence of increase in intracellular Ca^{2+} concentration on initiation of sperm motility. Possibly membrane hyperpolarization induces increase in intracellular Ca^{2+} from intracellular storage site [16] and the environment of sperm cells, resulting in initiation of sperm motility (Fig. 7) as well as in induction of other systems related to sperm motility.

Demembranated spermatozoa require cAMP for initiation of axonemal movement [108]. Cyclic AMP synthesized by adenylyl cyclase [114], which is known to be activated by increased intracellular Ca^{2+} in mammalian spermatozoa [69], induces activation of cAMP-dependent protein kinase (A-kinase) in the initiation of trout sperm motility [115]. The kinase phosphorylates and activates tyrosine kinase, since phosphorylation of Motility Initiating Phospho-Protein (MIPP) [77], with a molecular weight of 15,000 [112], occurs at tyrosine residue (Fig. 7). Several results suggest the activation of tyrosine kinase by activated A-kinase [63, 115].

MIPP was extracted from the semen of rainbow trout and the testis of chum salmon, and was isolated by affinity chromatographies [63 ,77]. The liver of chum salmon does not contain any phosphoprotein that corresponds to MIPP. Inhibitor of protein kinase, H-9 and a random copolymer of glutamate and tyrosine [63], and tyrosine-specific protein kinase, genistein [77], inhibit phosphorylation of the protein. During the purification procedure, MIPP migrates together with a 38 kDa phosphoprotein and a 45 kDa phosphoprotein, which may be the phosphorylating enzyme of MIPP (Fig. 1). Recent studies by western blotting showed that the 38 kDa and 45 kDa proteins are derived from the 72 kDa phosphoprotein, which may be the enzyme phosphorylating MIPP, e.g., tyrosine kinase (unpublished data).

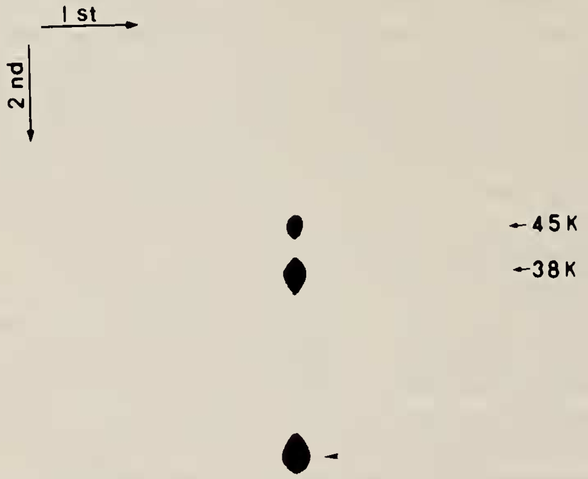


Fig. 1. Evidence for a phosphoprotein complex. The MIPP-complex which includes a 15 kDa motility initiating phosphoprotein (MIPP) and its phosphorylating activity i.e. tyrosine kinase, was purified from salmon sperm flagella using Blue Sepharose CL-613. The fraction from Sepharose was phosphorylated with radioactive ATP, and subjected to non-denaturing electrophoretic fractionation on 5% polyacrylamide gel. The first-dimensional gel was soaked in the sample buffer for SDS-polyacrylamide gel electrophoresis. Then electrophoresis in the second dimension was carried out in 12.5% gel. The autoradiogram is shown. The position of MIPP is indicated by an arrowhead. From Jin *et al.* [77].

Of 64 clones that were positive for a protein complex containing MIPP by ELISA, 11 clones were positive by western blotting assay [78] and three monoclonal antibodies, FMI7, FMI17 and FMI27, inhibited both the initiation of axonemal movement of the demembranated sperm and phosphorylation of MIPP (Fig. 2). Immunofluorescence staining of the demembranated sperm with the three monoclonal antibodies showed that the antigens were located in the basal region of the sperm flagellum (Fig. 3). An iontophoretic technique showed that when Ca^{2+} and cAMP were applied along the flagellar of intact and demembranated sperm, respectively, initiation of flagellar motility occurred when the second messengers were attached to the basal region of the flagellum (Okuno, Morisawa; unpublished data). Our studies by immunohistochemistry and iontophoresis strongly suggest that the cell signaling systems that are involved in the control of flagellar movement are located in the basal region of the flugellum in salmonid fishes (Fig. 7).

B Osmotic shrinkage or swelling of sperm mediating motility controlling systems in freshwater and marine teleosts and amphibians

Spermatozoa of marine teleosts, such as the puffer fish and flounder, and freshwater cyprinid teleosts, such as the carp, goldfish [107, 109, 127], zebrafish [167] and pejerrey [157], and of amphibians, such as toads, frogs [74] and newt [60] are quiescent in solutions with or without electrolytes when the osmolality is isotonic with that of the seminal plasma (almost 300 mOsm kg^{-1}), but become motile when the semen is diluted with a hypotonic solution for freshwater teleosts and amphibians and with a hypertonic solution for marine teleosts. Osmolality also has the same effect on the motility of lamprey sperm [83]. The osmolality-dependent initiation of sperm motility also mimics that in the environment on spawning of these animals in natural conditions.

Conversion of motility to immotility and vice versa, of sperm in freshwater cyprinid teleosts and marine teleosts occurs on changing the external osmolality [167]. As shown in Figure 4A, increase and decrease in osmolality of the environment caused reversible initiation and termination of sperm motility in a marine teleost, the puffer fish. Conversely, decrease and increase in osmolality caused reversible initiation and termination of sperm motility in the freshwater teleost, the zebrafish. Similar changes were observed in sperm of an amphibian, *Xenopus* (Inoda, Morisawa; unpublished data). These results suggest that osmolality reversibly regulates sperm motility. Furthermore, motility of demembranated sperm of freshwater and marine teleosts can be

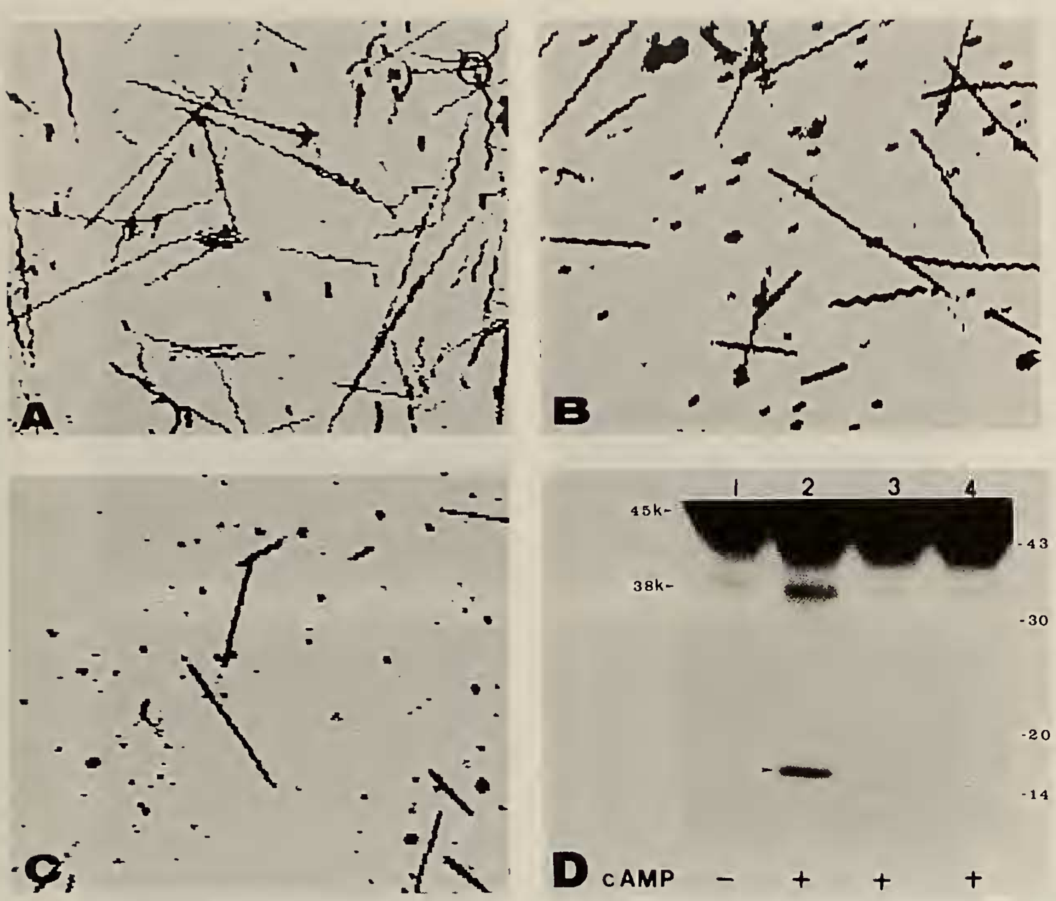


Fig. 2. Inhibition of trout flagallar motility and phosphorylation of the MIPP-complex by monoclonal antibodies to the MIPP-complex. Demembranated spermatozoa of rainbow trout obtained by Triton X-100 extraction were treated with normal mouse serum (A), monoclonal antibody from clone 2E11.2.2 (B) and monoclonal antibody from clone 2E7.1.1 (C), and their motility in reactivating solution containing cAMP and ATP was photographed. The image of tracks of heads which are driven by axonemal movement were observed as lines under a microscope. Motility of sperm in A, B, and C is 70%, 29% and 12%, respectively. For (D), the MIPP-complex partially purified from chum salmon sperm was preincubated with monoclonal antibodies and the cAMP-dependent phosphorylation was monitored by conventional procedures. Lane 1, negative control without cAMP; lane 2, positive control with cAMP; lane 3, after preincubation with clone 2E7.1.1; lane 4, after preincubation with clone 2E11.2.2. No phosphorylation of 15kDa MIPP(arrowhead) or 38 kDa protein was detected in lane 3 and 4. From Jin *et al.* [78]

manipulated repetitively by changing the environmental ion concentration (Fig. 4B). The demembranated sperm of both a marine teleost, the puffer fish and a freshwater teleost, the zebrafish, are immotile in reactivating solution containing 150 mM K^+, which may be equivalent to their intracellular K^+ concentration. Increase and decrease, and decrease and increase in the K^+ concentration of the reactivating solution caused initiation and termination of the motility of demembranated sperm of the puffer fish and zebrafish, respectively. Measurement of the intracellular K^+ concentration $[K^+]_i$ of sperm of a marine teleost, the puffer fish, by the null point analysis with a K^+/H^+ ionophore, nigericine [6], showed that $[K^+]_i$ increased during the initiation of sperm motility at hypertonic osmolality. These findings suggest that increase in osmolality around sperm causes increase in $[K^+]_i$ which affects the flagellar axoneme, resulting in initiation of flagellar motility. In hypertonic solution, puffer fish spermatozoa appear shrunken under a microscope. Moreover, the volume of the sperm pellet is less in hypertonic solution than that in isotonic solution, suggesting that increase in $[K^+]_i$ is due to decrease in cell volume by extrusion of water in hypertonic conditions. In the freshwater teleost, the carp, the cell volume increases in hypertonic conditions [109] and $[K^+]_i$ may possibly decrease.

The change of external osmolality is obviously harmful to cell structures and functions. In general, hypo- or hyperosmolality causes disruption of cells by their swelling or shrinkage. The volume of swollen or shrinken blood cells and bacteria is readjusted by cell signaling systems [57]; decrease in the cell volume in thymic lymphocytes after swelling is accomplished by loss of KCl and water from the cells by Ca^{2+}-activation of ion channels. In sperm of marine invertebrates, however, swelling of the head is observed immediately after immersion in hypotonic seawater and then structures are completely destroyed. Diluted Ringer solution causes spiralling of mammalian sperm tails and loss of

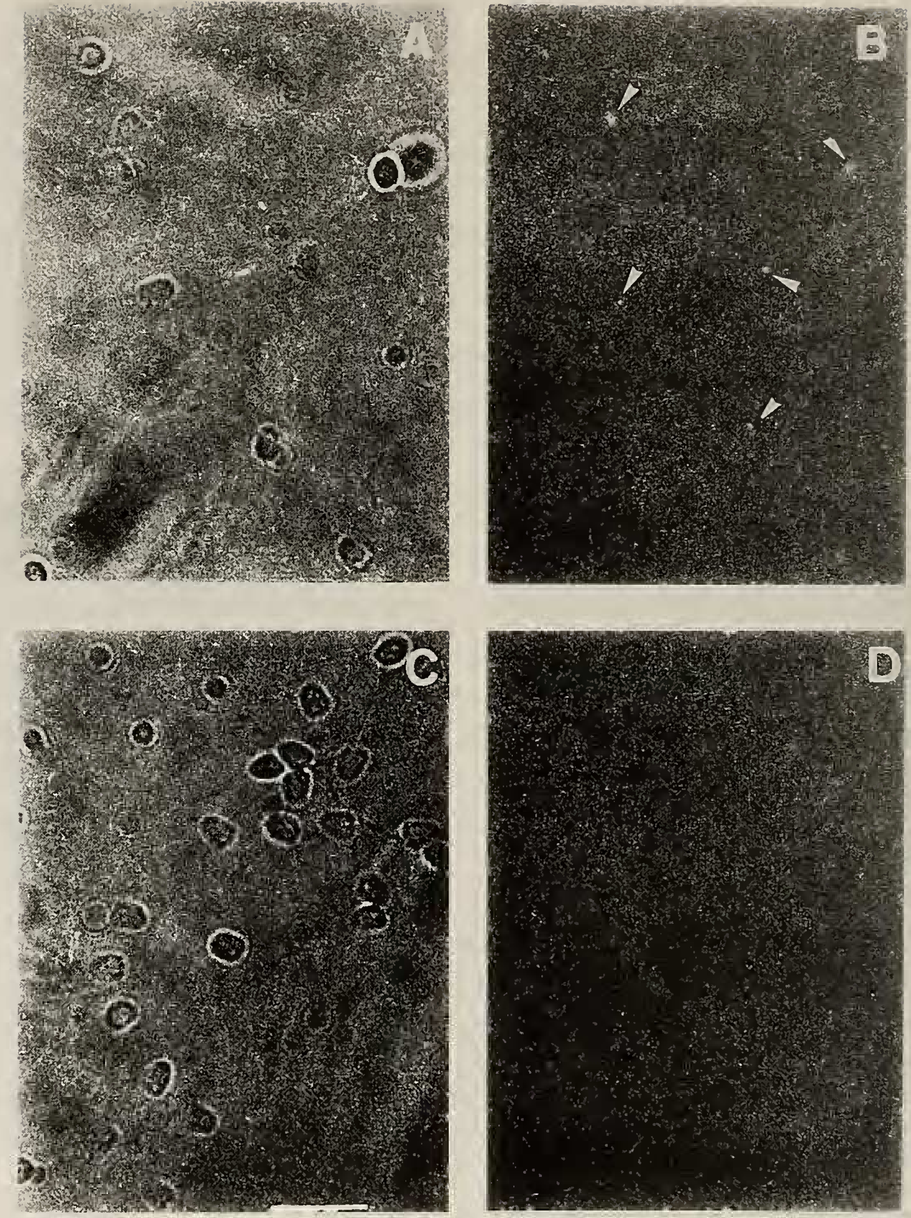


Fig. 3. Evidence for the presence of the MIPP-complex in the basal region of the flagellum. Rainbow trout spermatozoa were attached to the surface of a glass slide and then permeabilized with 0.05% Tween 20. Samples were treated with monoclonal antibody FM17 (A, B) or control mouse gammma globulin (C, D). FITC-labeled horse anti-mouse IgG was used as the secondary antibody. A, C; phase-contrast images. B, D; fluorescence images of the same fields as A, B, respectively. The bar represents 10 μm. FITC fluorescence is detected in the basal region of flagella(white arrowhead) in B. From Jin *et al.* [78].

motility [46, 47]. Swollen or shrunken sperm heads and fragments of tails of sperm of amphibians and marine teleosts were observed in hypo- or hyper-tonic solutions, respectively. Nevertheless, change in external osmolality acts as a trigger for cell signaling systems in sperm. At spawning, mature males and females of fishes come close to each other and release sperm and eggs simultaneously [155]. The duration of sperm motility of teleosts is generally short [107]. Thus, during the short period of approach of sperm to eggs, shrinkage or swelling of sperm cells by change of the environmental osmolality occurs and concomitantly increase or decrease in $[K^+]_i$ occurs (Table 2). The drastic changes of homeostasis in sperm cells may change the mechanochemical properties of the motile apparatus of the flagellum axoneme, resulting in initiation of sperm motility in freshwater and marine teleosts and amphibians.

It is still unknown how increase or decrease in $[K^+]_i$ induces the cascade of events for initiation of sperm motility in freshwater cyprinid and marine teleosts and amphibians, but possible contributions of Ca^{2+} and intracellular pH, $[pH]_i$ have been proposed in marine teleosts [127]. In the two species of puffer fish, *Takifugu niphobles* and *T. pardalis*, a chelating agent, EGTA, had no influence on the osmolality-dependent initiation of sperm motility, suggesting that ex-

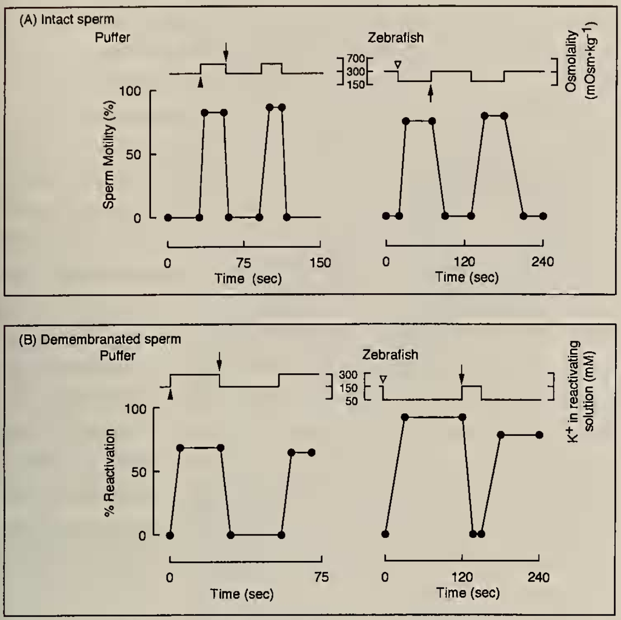


FIG. 4. Repetitive induction of motility in intact and demembranated spermatozoa of a freshwater cyprinid and marine teleost. (A) Spermatozoa of the puffer fish and zebrafish diluted with isotonic 150 mM NaCl solution (300 $\mathrm{mOsmKg^{-1}}$) were completely immotile. But when 5 M NaCl was added to puffer fish sperm to increase osmolality (700 $\mathrm{mOsmKg^{-1}}$, arrowhead) or Hepes buffer was added to zebrafish sperm to decrease the osmolality (150 $\mathrm{mOsmKg^{-1}}$, white arrowhead), the spermatozoa became fully motile. The motility was lost on return of the spermatozoa to solution of isotonic osmolality (arrows). (B) Demembranated sperm of the puffer fish and zebrafish suspended in reactivating solution containing 150 mM K^+ which is equivalent to the $[K^+]_i$ showed no axonemal movement. Upon transfer of the immotile sperm to reactivating solution containing 300 mM high K^+ (puffer fish, arrowhead) or 50 mM low K^+ (zebrafish, white arrowhead), the sperm axoneme became motile. In both case, motility stopped on return to 150 mM K^+ (arrows). Note that the initiation and termination of activity of both intact and demembranated sperm was reversible on changing the external osmolality and K^+ concentration of the reactivating solution. From Takai and Morisawa (original figure).

tracellular Ca^{2+} has no effect on motility initiation. Increase in intracellular Ca^{2+}, $[Ca^{2+}]_i$, on initiation of sperm motility in hypertonic solution was detected by measuring the fluorescence of Quin-2 loaded in sperm as the Quin-2/AM form. The increase occurred in the absence of external Ca^{2+}. In isotonic solution spermatozoa were immotile, however, when Ca^{2+} was introduced into the sperm by treatment with Ca^{2+} ionophore, motility was initiated. Further studies on the role of Ca^{2+} in osmolality-dependent motility initiation are necessary.

Decrease in $[pH]_i$ on initiation of trout sperm motility was reported, but artificial decrease of $[pH]_i$ did not activate motility, suggesting that $[pH]_i$ is independent of motility initiation in salmonid sperm [15, 52, 113]. The possible role of $[pH]_i$ in the initiation of sperm motility was suggested in marine teleosts. Motility of spermatozoa of the flounder, *Kareius bicoloratus*, and the puffer fish, *T. niphobles*, was initiated at isotonic osmolality when $[pH]_i$ was increased by NH_4Cl or $(NH_4)_2SO_4$ [127]. Furthermore, increase and decrease of the pH of reactivating solution caused the initiation and termination of motility in demembranated sperm (data not shown), suggesting that $[pH]_i$ is another factor regulating the intracellular signaling system for the initiation of sperm motility. However, it is still unknown, whether activation of the Na^+/H^+ exchange mechanism by osmotic effects on the plasma membrane of teleost sperm occurs as reported in blood cells [57]. In addition, controversial results of increase or no increase of $[pH]_i$ on osmolality-induced initiation of motility have been obtained using different pH-sensitive fluorescent dyes [127, 167].

III SPERM ACTIVATION AND CHEMOTAXIS INDUCED BY SIGNALS FROM THE EGG

After initiation of sperm motility by the signals of change in the ionic or osmotic environment, the motility is sometimes increased in response to secretions released from the eggs or the female reproductive organs. Sperm chemotaxis toward eggs occurs under the influence of a chemo-attractant released from the egg to complete fertilization. In some cases, such as in the herring and *Ciona*, these secretions may act not only to increase motility and chemotaxis, but also to trigger initiation of motility if this is not initiated by change of the environment upon release from the genital pore.

A Sperm motility initiating and activating peptides in fishes

Teleost spermatozoa have certain advantages for use in studies on sperm-egg interaction at fertilization. They lack an acrosome, and so signal transduction mechanisms in sperm activation and initiation of motility do not overlap acrosome-related events. Sperm activation and chemotaxis occur near the egg micropyle, which is a hole perforated the chorion at the animal pole of the egg through which the sperm move toward the egg, and the plasma membranes of the sperm and egg fuse directly with each other at fertilization.

The motility of spermatozoa of three species of Japanese bitterlings is initiated in fresh water and then the spermatozoa move by rotating toward the micropyle where they accumulate and then disperse. The sperm accumulating substance that is present and diffuses out near the micropyle is a dialyzable substance of low molecular weight that is resistant to heat and alcohol, but destroyed by trypsin treatment [162–165]. A jelly-like flask covering the animal pole of the lamprey egg attracts and aggregates spermatozoa [151]. The spermatozoa move quickly and aggregate in egg water, suggesting that the sperm attracting and aggregating substance is released from the egg, although no studies on its chemical nature and distribution have been reported.

Spermatozoa of the herring, *Clupea palasii*, that are immotile or only slightly motile in diluted seawater or in isotonic solution, start to move actively when they come in contact or nearly in contact with the egg surface [192, 197] or near the egg surface [116]. A few spermatozoa swim actively at the vegetal hemisphere and in regions far from the eggs [116, 192]. The factor for activation of sperm requires Ca^{2+} and is supposed to be a protein [193]. As shown in Figure 5, herring spermatozoa are completely immotile in a solution isotonic to the seminal plasma, but about 20% of the sperm start swimming at a speed of about 170 μm/sec in hypertonic 60–70% seawater. The number of motile sperm and their speed of swimming (about 200 μm/sec) increase when egg seawater is added, suggesting that a sperm activating substance is released from the eggs [116]. This substance was purified from egg water by gel filtration and isoelectric focusing. In this way five different herring sperm-activating peptides (HSAPs) were obtained with isoelectric points of 4.8, 4.9, 5.0, 5.1 and 5.4. The molecular weight of the major peptide (pI=5.1) was found to be 8.1 kDa by mass spectrometry [128].

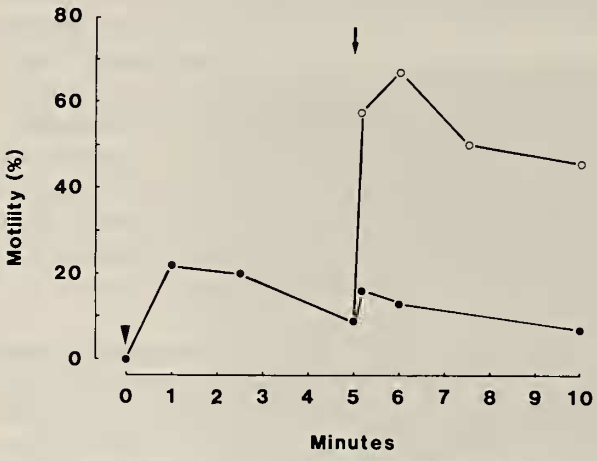


Fig. 5. Initiation and activation of motility of herring sperm. Herring eggs were incubated in seawater for 1 hr at 4°C. The supernatant named egg water, was obtained by low speed centrifugation. Semen was diluted with 80% seawater (arrowhead) and sperm motility was monitored (●). Then egg water (○) or 80% seawater (●) was added (arrow), and monitoring of motility was continued. Motility was initiated in about 20% of the spermatozoa on exposure to seawater. Motility was initiated in almost all spermatozoa by exposure to egg factor. From Morisawa *et al.* [116]

Detailed observations on sperm behavior around the micropyle area of a herring egg were reported by Yanagimachi *et al.* A preparation of spermatozoa in which less than 0.1% were moving in diluted 50% seawater began to move actively as they came in contact with the micropyle area. In this experiment, one egg was placed in 10–12 ml of Ringer solution [197], which may cause the dispersion and dilution of HSAPs released from the egg in the large volume of Ringer solution, and so sperm activation around the egg may not have been detected. In the author's experiment, washing of 70 herring eggs with 6 ml of isotonic 300 mM NaCl solution resulted in depletion of HSAPs activity of the eggs (unpublished data).

The radially arranged grooves located around the egg micropyle of the freshwater cyprinid fish Rosy-barb, *Barbus conchonius*, are reported to guide spermatozoa into the micropyle [1]. The micropyle area of the herring egg chorion possesses a factor which enhances sperm motility and attracts or guides spermatozoa into the micropyle [197]. The activity of the factor is lost on treatment with trypsin or acidic sea water. Pillai *et al.* [139] purified the factor, sperm motility initiating factor (SMIF) from an acid seawater extract of herring eggs and identified it as a glycoprotein with molecular weight of 105 kDa. HSAPs and SMIF may function during approach of herring sperm to the egg at fertilization. After partial initiation of motility by increase in the osmolality around the spawned sperm, HSAPs released from eggs cause further motility initiation and activation of immotile sperm, resulting in fully motile spermatozoa. When the activated sperm come in contact with the micropyle area, SMIF further activates sperm motility and guides sperm into the micropyle (Table 2) to complete the fusion of the plasma membrane of gametes.

B Sperm activation and chemotaxis in hydrozoans and ascidians

The first observation of sperm chemotaxis in animals was that of sperm accumulation near the germinal vesicle of the egg in the hydromedusa, *Spirocodon saltatrix* [42]. Evidence for sperm chemotaxis was accumulated in the Hydroids, *Campanularia, Tubularia, Gonotyrea* and *Clava etc.*, in hydromedusa (for review, see ref. [99]). In siphonophores [23] as well as in a leptomedusae [95], spermatozoa are attracted or agglutinated at a point on the egg surface that is the site of emission of the polar bodies. The diameter of circular trajectories of sperm, progressively decreases near the cupule, the site of sperm attraction and entrance in siphonophores or the tip of pipette containing cupule extract.

Ciona sperm exhibit both typical activation of motility and chemotaxis under the influence of eggs [93, 97]. Our recent studies on the both events in the ascidians *Ciona intestinalis* and *C. savignyi* showed that a sperm-activating and -attracting factor (SAAF) is released from the egg itself (Fig. 6) rather than the follicle cells [93], and that the sperm-activating and -attracting activities of the egg disappear at the time of completion of fertilization [198].

There are conflicting reports with regard to the site of sperm entrance into the egg in ascidians. In *Styela*, the spermatozoon enters near the vegetal pole of the egg [34]; in *Phallucia*, the spermatozoon enters near the animal pole and then is carried to the vegetal pole area by ooplasmic segregation [154]; *Molugla* sperm bind at any site on the egg surface and then are carried to the vegetal pole area [149]. In *Ciona*, spermatozoa are attracted to the vegetal pole of a naked egg suggesting that the attractant is released from the vegetal pole (Fig. 6, see ref. [198]). The sperm-activating and -attracting substance is a dialyzable, heat-stable, probably nonproteinacious small molecule and controls both activation and attraction by different mechanisms [198]).

The second messengers, cAMP and Ca^{2+} have received

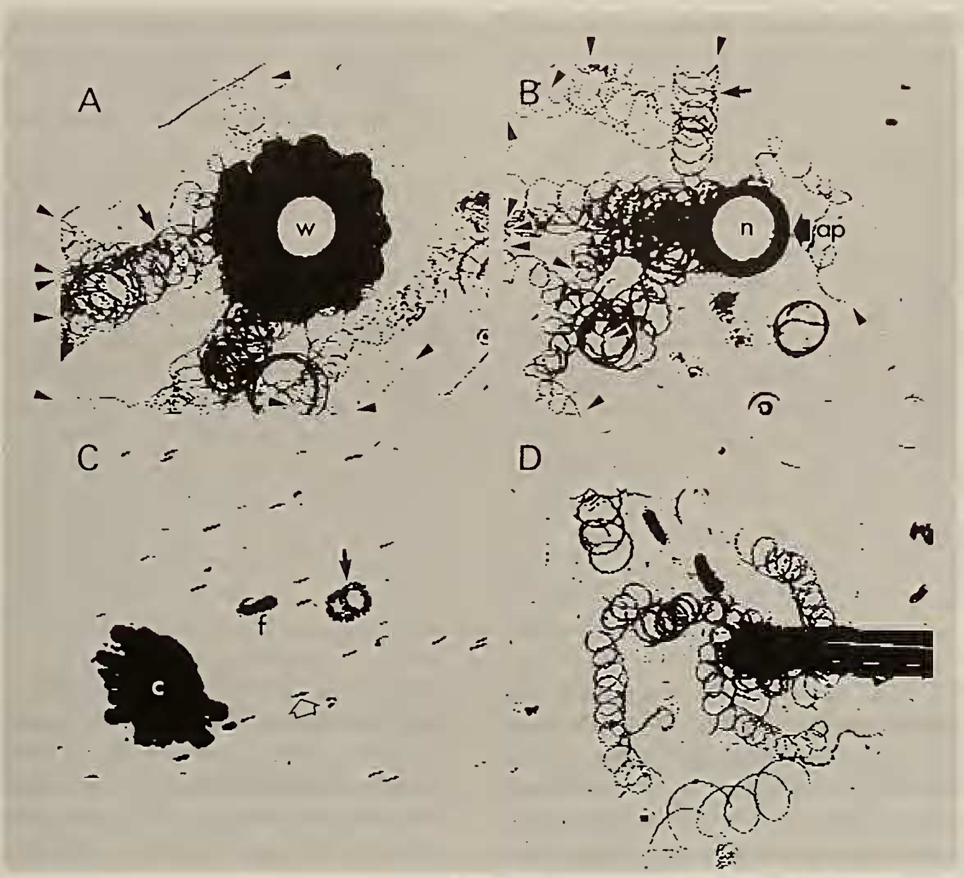


FIG. 6. Sperm activation and attraction activities of *Ciona* eggs and purified sperm-activating and -attracting factor (SAAF). A, B and C: A follicle cell (f), chorion (c), and naked egg (n) were separated from a whole egg (w) and placed in sperm suspension. (A) Sperm are seen around the whole egg. (B) Sperm are seen on the left side of the naked egg. Polar bodies (ap) appeared on the right portion (arrow) suggesting that sperm were attracted toward the vegetal pole of the egg. (C) Sperm are seen to be immotile around the follicle cell and chorion. D: The tip of a capillary containing SAAF inserted into a sperm suspension. The sperm were attracted toward the SAAF moving with circular trajectories. Sperm trajectries are shown as continuous circular lines. Black spots indicate the heads of immotile sperm. Arrowheads show the beginning points of sperm trajectories. From Yoshida *et al.* [198, 199].

considerable attention as regulators of activation and attraction of *Ciona* sperm. The Ca^{2+}-chelator EGTA suppresses sperm motility of *Ciona* and addition of Ca^{2+} results in activation of motility, suggesting the participation of Ca^{2+} in sperm activation [111]. Furthermore, a recent study by Yoshida *et al.* [199] showed that flunarizine, a T-type (transient type) Ca^{2+}-channel selective antagonist, inhibits activation of *Ciona* sperm by SAAF, and that sperm activation does not occur and cAMP is not synthesized in Ca^{2+}-free seawater. The level of cAMP increase concomitantly with the activation of sperm motility in the presence of Ca^{2+}. Thus it is thought that SAAF causes Ca^{2+} influx through T-type Ca^{2+}-channels and synthesis of intracellular cAMP. The cAMP induces motility of the axoneme, the motile apparatus in the flagellum, resulting in activation of *Ciona* spermatozoa (Fig. 7). Activation of axonemal movement by cAMP is known to occur in parallel with cAMP-dependent phosphorylation of proteins including dynein heavy-chain components [19, 135]. Therefore, one pathway regulating sperm motility in *Ciona* occurs along the whole length of the sperm flagellum except for limited portion, although another pathway such as that known in salmonid fishes (Fig. 7) is still possible.

The chemotactic behavior of siphonophore sperm cannot be observed in Ca^{2+}-free seawater and the wave form of sperm flagella is symmetrical in Ca^{2+}-free or low-Ca^{2+} seawater in the presence of sperm attractant or the calcium ionophore A23187 [39]. The wave form becomes asymmetrical when the Ca^{2+} concentration is increased [38], suggesting that the chemoattractant from the cupule might modulate flagellar asymmetry and generate the chemotactic response (Table 2). The requirement of Ca^{2+} for sperm chemotaxis toward the egg was also observed in an ascidian [93]. SAAF, the sperm-attracting and -activating factor of *Ciona*, also requires Ca^{2+} for the modulation of chemotactic behavior of the sperm as well as for induction of activation. However, cAMP, an intracellular factor for activation of *Ciona* sperm, is independent of sperm chemotaxis, because increase in cAMP by treatment of sperm with teophilline, a phosphodiesterase inhibitor did not induce chemotaxis [199]. Chemotaxis of *Ciona* sperm does not occur in Ca^{2+}-free seawater in the presence of SAAF, but spermatozoa are attracted toward the tip of a capillary containing SAAF by

Table 1. Properties of sperm chemoattractants in marine invertebrates

	MW range (kDa)	Heat Stability	Protease Sensitivity	Species-specificity	Ref.
Hydrozoans					
Anthomedusae	5–14	+	?	+	92, 96
(Sessile)	<1	+	+	−	92, 96
Limunomedusae	>5	+	?	+	92, 96
Leptomedusae	<1	+	?	+	92, 97
Siphonophore	20–25	+	+	+	23, 40
Coral	<0.5	(lipid like substance)		+	32, 33
Mollusca					
Chiton	?	?	?	−	94
Echinoderms					
sea urchin	1–2	+	+	+	160, 187
starfish	10–12	+	+	+, −	140
brittle star	?	?	?	+	98
sea cucumber	?	?	?	−, +	98
Chordata					
Ciona	<12–14	+	−	−	99, 199

progressive decrease in the diameter of the sperm track with proximity to the attractant source (Fig. 6). Generally, symmetry of the flagellar wave form generates a linear sperm trajectory, whereas flagellar asymmetry generates narrow and circular trajectories. A high Ca^{2+} concentration causes asymmetrical waves in demembranated sea urchin spermatozoa [18, 22]. From the data on Ca^{2+} control of flagellar asymmetry in siphonophore sperm chemotaxis, Cosson *et al.* [37] proposed the following hypothetical model for the generation of chemotaxis: "When spermatozoa swim close to a gradient of attractant, binding of a few attractant molecules to the sperm cells induces a transient calcium influx which, in turn, increases flagellar asymmetry and narrows the sperm trajectory". In *Ciona*, the motility of intact sperm is suppressed by Ca^{2+} channel antagonists in the presence of SAAF. Thus Ca^{2+} influx through Ca^{2+} channels by the action of SAAF may increase the asymmetrical beating of the axoneme resulting in the induction of chemotactic behavior of spermatozoa (Fig. 7).

In animals, the chemical nature of sperm attractant is considered to be a peptide or protein with a molecular weight varying from 1 to 2 kDa [158] in the sea urchin to 20–25 kDa in siphonophores [40]. Sperm chemotaxis is highly species-specific, whereas the sperm chemoattractants from three *Tubularia*, coral and chiton have no species specificity. In *Ciona intestinalis* and *C. savignyi*, the egg of each species can activate and attract the sperm of both species, suggesting no species specificities in SAAF of *Ciona* (Table 1). SAAF is a dialyzable, small molecular weight compound of less than 12–14 kDa that is heat-stable and resistant to proteases such as trypsin, thermolysin, and pronase E, suggesting that it may not be a protein. Recently, coral sperm chemoattractant was also proposed to be a low molecular weight nonproteinacious substance [32, 33]. During purification of SAAF by column chromatographies, the sperm-activating and -attracting activities are always co-eluted (unpublished data). Furthermore, spermatozoa exhibit "turn", which is an index of chemotactic behavior of sperm, near the purified SAAF. Thus it seems that one kind of molecule (SAAF) mediates two different pathways: Ca^{2+}- and cAMP-dependent sperm activation, and sperm attraction which is regulated by Ca^{2+} alone.

IV INITIATION AND ACTIVATION OF SPERM MOTILITY AND SPERM CHEMOTAXIS IN OTHER ANIMALS

A Sea urchins and other invertebrates

In sea urchin, alkalization of the inside of sperm cells by efflux of CO_2, a volatile inhibitor of sperm motility [79] at spawning and efflux of H^+ by Na^+/H^+ antiport [125] induce activation of dynein ATPase, resulting in the initiation of sperm motility [26]. Concomitantly with energy consumption after the initiation of sperm motility, ATP is supplied through the phosphorylcreatine shuttle [178] to confer the sperm with full motility (Fig. 7). Increase of $[pH]_i$ by a factor released from egg jelly also induces activation of respiration and motility of sea urchin sperm.

The first evidence for sperm activation in invertebrates was the observation of increase in sperm motility of the annelid *Nereis* and the sea urchin, *Arbacia* in egg seawater containing secretions of eggs [86], but demonstration of a sperm activating substance in egg jelly was established by the studies of Ohtake [129, 130]. Sperm activating peptides with a sequence of 10 amino acids were purified from the egg jelly of the sea urchin, *Hemicentrotus pulcherrimus* [159] and *Strongylocentrotus purpuratus* [51, 58] and named as speract. Numerous peptides such as resact in Arbacia punctulata [160] and mosact in *Clypeaster japonicus* [161] *etc.* were subsequently purified, and their amino acid sequences were determined. They are given a systematic nomenclature based on the taxonomic order of the species by Suzuki [158]; SAP-I in the order Echinoida; SAP-II in the order Arbacioida; SAP-III in the order Clypeasteroida, and SAP-IV in the Diadematoida.

Extensive studies on signal transduction occurring in activation and sperm chemotaxis in sea urchin sperm have been conducted by Garbers and his colleagues (Fig. 7). Receptors for speract and resact which are located in the plasma membrane of sperm were identified and characterized as acidic glycoproteins with molecular weights of 77 kDa and 160 kDa, respectively [11, 43, 44, 152]. A 160 kDa protein from *A. punctulata* was identified as guanylyl cyclase [152]. Guanylyl cyclase is considered to be associated with the receptor of speract [152, 177] since a large amount of the enzyme is present in *S. purpuratus* testis [177] and is activated by speract [14]. Guanylyl cyclase itself is the receptor for atrial natriaretic peptide (ANP) [168] which has the physiological effects of causing natriuresis, diuresis, *etc*. The receptors of both ANP and resact are proteins penetrating the plasma membrane. The amino acid sequences of the receptors for resact and ANP have similarity in their intracellular portions, but little in their extracellular portions [25]. The fact that the receptors of sperm activating peptides act as receptors for ANP in mammalian tissue, indicates that similar cell signaling mechanisms have different physiological functions in different tissues or organs throughout the animal kingdom.

Upon exposure to speract or resact, guanylyl cyclase in sperm is stimulated and the cGMP level increases [12, 50, 142]. Guanylyl cyclase is predominantly in a high molecular weight and phosphorylated active form, and is converted to a low molecular weight, dephosphorylated form with low activity on exposure to speract and resact [13, 143, 188]. The peptides also induce phosphorylation of membrane proteins in the presence of GTP [14]. From these findings, it is possible that binding of the peptides to the receptor which is located close to guanylyl cyclase causes elevation of cGMP level, resulting in phosphorylation of proteins. The guanylyl cyclase becomes a dephosphorylated inactive form.

Speract and monesine, an ionophore capable of causing Na^+/H^+ exchange, induce both Na^+/H^+ exchange and activation of spermatozoa [59, 144]. The activation of the Na^+/H^+ exchange by speract occurs through hyperpolarization of the plasma membrane caused by K^+ efflux [85]. It is also suggested that Ca^{2+} influx occurs through activation of Na^+/H^+ exchange by speract [150]. Recently, Cook and Babcock proposed that cGMP activates a K^+ channel with the resulting cascade of hyperpolarization of the plasma membrane, activation of Na^+/H^+ exchange and increase in $[pH]_i$. The alkalization causes activation of adenylyl cyclase and elevation of cAMP which induces Ca^{2+} influx through activation of the Ca^{2+}-channel [35, 36]. Ca^{2+} and phosphorylation of protein cause downstream events for initiation and activation of sperm motility (Fig. 7). Recently, receptors for sperm activating peptides were also identified in Japanese sea urchins, *Hemicentrotus pulcherrimus, Clipeaster japonicus, etc.* [61, 200] and the roles of membrane hyperpolarization, Ca^{2+} and $[pH]_i$ in sperm functions [62, 66] were investigated in the species.

Ca^{2+} requirement for sperm chemotaxis in hydrozoans and ascidians has been discussed in Chapter III B. Activity as a chemoattractant has been reported only for resact among the egg peptides of sea urchins [187]. Speract has no chemotactic activity. Extracellular Ca^{2+} is required for this activity. In the case of resact-induced chemotaxis, the track diameter of sperm trajectories increases and the sperm swim in straighter trajectories, somewhat different from the sperm chemotaxis reported in siphonophores [39] and *Ciona* [198]. In addition to functioning in sperm activation and chemotaxis, speract can potentiate the effect of a high molecular weight acrosome reaction inducing substance from egg jelly through increase in the cAMP level [191]. Both paths of cell signaling mechanism, i.e. cGMP-dependent protein phosphorylation and increase in $[pH]_i$ and $[Ca^{2+}]_i$ finally cause activation of sperm, such as increase in respiration and motility, chemotaxis and induction of the acrosome reaction (Fig. 7).

Cyclic AMP-dependent phosphorylation plays a key role in the activation of sperm motility in the sea urchin [20, 76] and starfish [76]. According to Ishiguro *et al.*, a fraction obtained from a Triton X-100 extract of sea urchin or starfish sperm, contains cAMP-dependent protein kinase and a protein factor associated with the enzyme. The phosphorylated form of the protein factor is a prerequisite for reactivation of demembranated sperm, suggesting the requirement of phosphoprotein for the initiation and activation of sperm motility [76]. Gibbons and Gibbons [54] reported that addition of cAMP has no effect on motility of the demembranated sperm. Cyclic AMP sometimes does not affect demembranated sperm prepared by Triton X-100 treatment from very fresh sea urchin spermatozoa. However, preincubation of sperm at room temperature made them susceptible to cAMP-dependent reactivation, indicating that dephosphorylation occurs during demembranation of sperm, which leads to their quiescence [76]. Phosphorylation of proteins by the cAMP system may have an important role in regulation of axonemal movement in echinoderms.

An extensive cascade system for the sperm functions was demonstrated in silkworm, *Bonbyx mori* by Osanai and his colleagues (Table 2). During the process from formation of spermatozoa to fertilization; (a) spermatogenesis in the testis, (b) transfer of two types of spermatozoa, apyrene and eupyrene spermatozoa, to the vesicula seminalis and their storage in it, (c) ejaculation from male glands into the busa copulatrix of a female to form the spermatophore, (d) disappearance of apyrene spermatozoa, and completion of fertilization, “initiatorine” a specific endopeptidase acts as the trigger for sperm maturation and initiation of arginine degradation cascade for an energy-yielding [136]. Apyrene spermatozoa require the initiatorine and a cyclic nucleotide, cAMP or cGMP for activation of flagellar movement [137], suggesting that second messenger-dependent cell signaling mechanisms and enhancement of energy metabolism have key role for the regulation of sperm function in insect.

B *Mammals and chickens*

Control of the motility of mammalian spermatozoa occurs at two critical stages. One is at ejaculation; when motility is initiated for the first time in the life span of the sperm. The other control occurs when the sperm reach the upper portion of the female reproductive tract; where sperm motility is stimulated, or "hyperactivated", and sperm become sensitized to stimuli for inducing the acrosome reaction through the process of capacitation which enables them to penetrate the zona pellucida surrounding the egg.

In marine invertebrates such as the sea urchin, and in teleosts, such as the trout, puffer fish, and zebrafish, spermatozoa are completely quiescent in undiluted semen. However, the degree of motility of mammalian sperm in undiluted semen varies with the species. Samples from the distal part of the cauda epididymis contain many motile sperm [123] in the rabbit (25%), bull (35%) and especially man (60%). Sperm with low motility have also been found in this region [123] in the rat (2%), mouse (1%) and hamster (1%). It is uncertain whether the observed motility represents the true *in situ* motility within the epididymis. Motility is initiated in a considerable proportion of the spermatozoa on mixing with the seminal plasma flushed from the accessory glands and ejaculated into the female reproductive tract.

Nevo [124] showed that CO_2 completely inhibited the motility of bull sperm, suggesting that an anaerobic CO_2-rich condition in the male reproductive tract suppresses sperm motility before ejaculation (Table 2). Cascieri *et al.* [24] found that bull sperm are already partially motile before dilution and suggested that reduction of mechanical restraint through dilution is a factor for initiation of sperm motility. Storey [156] reported that some motile sperm can be obtained from the epididymis of the rabbit, and suggested that initiation of sperm motility at ejaculation is caused by both release from physical restraint and access to oxygen. The motility of rat sperm is suppressed by the restraint of a mucin-like glycoprotein, named immobilin present in epididymal fluid, and release from the restraint at ejaculation is proposed to be a factor for motility initiation [183]. Glycerylphosphocholine [181], carnitine [65], and a proteinaceous factor [182] have also been proposed as factors for restrain of rat sperm.

Stimulatory effects of Ca^{2+} (e.g. [120]) and effect of cyclic nucleotides (e.g. [75, 101, 122, 153, 186]) on the motility of many mammalian spermatozoa have been reported. The cyclic AMP level is low in immotile sperm but rapidly increases on initiation of motility. Furthermore, demembranated mammalian sperm show initiation of motility in the presence of cAMP in reactivating solution [75, 87, 101, 190]. There may be several pathways and following system for induction of sperm motility is possible. The epididymal fluid contains phosphodiesterase and a little Ca^{2+}, thus spermatozoa are immotile or slightly motile in the epididymis where they have a low intracellular cAMP content and low external Ca^{2+} level. In some mammalian species whose seminal plasma has a high Ca^{2+} content [123, 173], spermatozoa become motile on supplementation of Ca^{2+} from the seminal plasma at ejaculation, which causes rapid cAMP synthesis to induce motility.

A stimulatory effect of sodium bicarbonate on porcine spermatozoa in the seminal plasma has also been noted. A low molecular weight sperm motility activating factor, which increases cAMP by activation of adenylyl cyclase, was found in the porcine seminal plasma [131] and was identified as $NaHCO_3$ by Okamura *et al.* [132]. $NaHCO_3$ activates adenylyl cyclase, motility, and respiration in the sperm of many mammalian species such as porcine, bovine, rat, mouse, dog and human sperm [132, 153]. Anion channel blockers enhance the accumulation of endogeneous HCO_3^-, and sperm are activated by inhibition of the efflux of HCO_3^- derived from CO_2, suggesting that intracellular HCO_3^- stimulates adenylyl cyclase [166]. The roles of proteases in the regulation of sperm motility have been studied extensively by Gagnon [49], and Inaba and his colleagues recently focused attention on the relation of proteasomes to sperm motility [70–73]. A trypsin-like proteinase which binds to the plasma membrane of epididymal sperm and inhibits adenylyl cyclase, and a proteinase inhibitor in the seminal plasma were recently reported by Okamura *et al.* [133]. They proposed cascade system for initiation of mammalian sperm motility: namely, suppression of proteinase activity by the seminal proteinase inhibitor at ejaculation causes activation of HCO_3^--stimulated adenylyl cyclase, resulting in the rapid synthesis of cAMP, a second messenger that proceeds the further cascade mechanism of sperm motility initiation (Fig. 7). Bovine sperm, in contrast, contain a trypsin-like protease that activates adenylyl cyclase [80].

Changes in external ionic conditions such as in Ca^{2+} and HCO_3^- level and subsequent elevation of the cAMP level appears to be a major signal pathway in the triggering system for initiation of motility in mammalian sperm (see above) as well as other species such as teleosts, tunicates and sea urchins (see Chapter II and Chapter III A). Increase in $[pH]_i$ is also a factor regulating mammalian sperm motility [7, 8]. A type II regulatory subunit of cAMP-dependent protein kinase (RII) [126], with a molecular weight of 55–56 kDa [17], is the target of phosphorylation mediated by cAMP. The 56 kDa phosphoprotein, i.e. RII in mammalian sperm, was named axokinin [174]. Calmodulin, on the other hand, is known to be a mediator of signal transduction in calcium-regulated flagellar motility through protein phosphatase activity [173]. The facts that the sperm RII related to the calmodulin-dependent protein phosphatase is present in mammalian sperm [175], and that RII and the protein phosphatase are associated with dynein ATPase activity [172], suggest that both cAMP-dependent phosphorylation and Ca^{2+}-calmodulin-dependent dephosphorylation pathways regulate the ATP-induced sliding of the microtubule and control the process of initiation of mammalian sperm motility (Fig.

TABLE 2. Factors for regulation of sperm motility in animals

Specie	Motility	External signal	Membrane response	Internal factor	Ref.
Siphonophore	chemotaxis	peptide	Ca^{2+} channel	Ca^{2+}	39
Horseshoe crab	initiation & activation	peptide Zn removal			27–29, 31, 180
Silkworm	activation	endopeptidase		energy yielding, cAMP, cGMP	136, 137
Sea urchins*	initiation	CO_2, Zn addition	Na^+/H^+ exchange	pH	30, 79
	activation	speract etc	ANP-receptor	cGMP, cAMP	45
	chemotaxis	resact	hyperpolarization	Ca^{2+}	45
Starfish	initiation	Zn removal			102
	chemotaxis	peptide			140
Ciona*	initiation	SAAF	Ca^{2+}-channel	Ca^{2+}, cAMP	111
	activation	SAAF	Ca^{2+}-channel	Ca^{2+}, cAMP	198, 199
	chemotaxis	SAAF	Ca^{2+}-channel	Ca^{2+}	198, 199
Herring	initiation & activation	osmolality, HSAPs HSAPs SMIF			116, 128, 139
	chemotaxis	SMIF			139
Salmonids*	initiation	K^+	hyperpolarization	Ca^{2+}, cAMP	78, 104, 107
FW teleosts	initiation	osmolality	cell swelling	K^+ decrease	107, 167
SW teleosts	initiation	osmolality	cell shrinkage	K^+ increase	107, 127, 167
Amphibians	initiation	osmolality			60, 74
Chicken	initiation	temperature	temperature pass Ca^{2+} influx	temp effect phosphoprotein	2–5
Mammals*	initiation	$HCO^-{}_3$, Ca^{2+}, CO_2	protease activity	Ca^{2+}, cAMP, pH	36, 131–133, 172, 189, 194
	hyperactivation			Ca^{2+}, cAMP, pH	
	chemotaxis	peptide			141, 201

* see detail; Fig. 7

7).

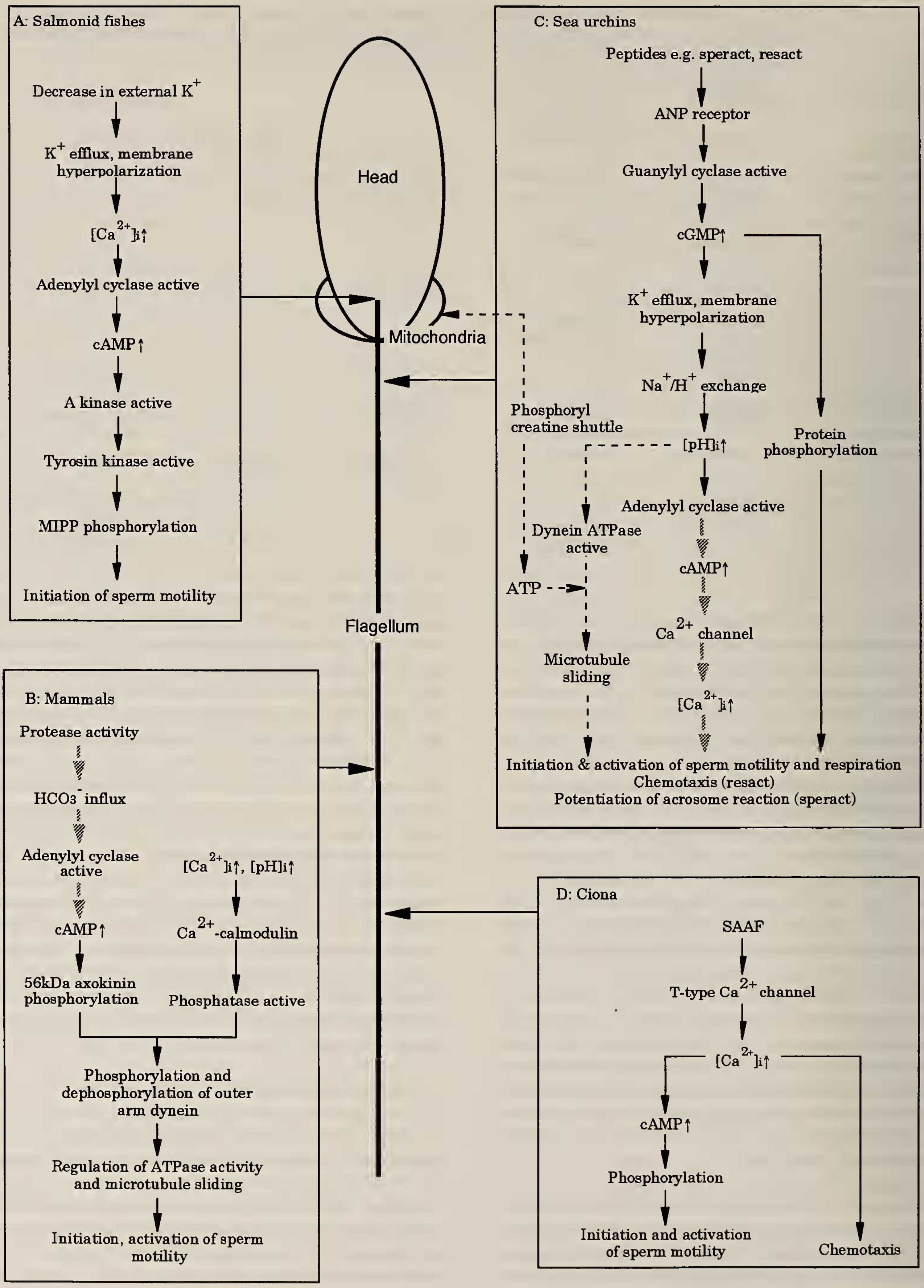
Hyperactivated motility of spermatozoa (hyperactivation) in mammals is a distinct, vigorous motility [194, 195] exhibiting high amplitude, crawling, serpentine, whiplash and frenzied or contorted flagellar movements, and is considered to play a significant role in fertilization. There are many reports on hyperactivated patterns of sperm flagella and their roles in fertilization, and there is some evidence for the contribution of a cell signaling system to the induction of hyperactivation (Table 2). Cyclic AMP synthesis in hamster sperm is enhanced when the sperm are incubated in a medium causing hyperactivation [121, 189]. The demembranated sperm exhibit hyperactivated motility in the presence of cAMP [101]. Ca^{2+} is related to the phenomenon [88, 138]. Probably calcium influx and a Ca^{2+}-calmodulin system are involved in hyperactivation of mammalian sperm [189, 196], but these possibilities have not yet been demonstrated conclusively.

N-Formylmethionyl peptides, which are chemoattractants for neutrophils and macrophages have a chemotactic effect on human spermatozoa [55]. Recent studies showed that human spermatozoa accumulate in follicular fluid *in vitro* [141] and that atrial natriaretic peptide, a known activator of particulate guanylyl cyclase, induces activation and attraction of spermatozoa [201].

Chicken sperm are unique in showing a response to temperature [2]. They are immotile at a body temperature of 40–41°C, but become motile on decrease in temperature to 30–35°C. The motility of demembranated chiken sperm also exhibit the same response to change of temperature, i.e. immotile and motile at a high and low temperature, respectively, suggesting that temperature change directly affects the flagellar motile apparatus, the axoneme to regulate sperm motility. Decrease in the intracellular Ca^{2+} level at 40°C immobilizes sperm and the subsequent addition of Ca^{2+} at 40°C causes initiation of sperm motility [176]. However, such effects of Ca^{2+} are not observed with demembranated sperm [3], suggesting that Ca^{2+} may not have a direct effect on the axoneme but that its effect is mediated by soluble substances that are removed in the demembranation procedure. Cyclic AMP also does not affect the motility of demembranated sperm [2], but a recent study suggested that cAMP-independent phosphorylation of a 43 kDa protein is necessary for induction of motility of chiken sperm at low temperature [4]. These results suggest that temperature controls the motility by at least two pathways: by a direct action on the motile apparatus, the axoneme, and by an indirect action through the change in $[Ca^{2+}]_i$ and the protein phosphorylation process (Table 2). The contribution of type 1 protein phosphatase to the initiation of chicken sperm motility was recently demonstrated [5].

C Roles of zinc in regulation of sperm motility

Zinc apparently contributes to the activation of respiration and motility in marine invertebrates such as the sea urchin, starfish and horseshoe crab and in mammals (Table 2). The motility of sea urchin sperm is not increased by addition of zinc or copper but is reduced by metal-chelating agents such as EDTA and amino acids [146]. Clapper *et al.* [30] reported that metal chelators and SH reagents cause removal of zinc from sperm, a delay in motility initiation, and a decrease of $[pH]_i$. Zinc is absorbed and the motility and


A: Salmonid fishes
Decrease in external K+
K+ efflux, membrane hyperpolarization
[Ca2+]i↑
Adenylyl cyclase active
cAMP↑
A kinase active
Tyrosin kinase active
MIPP phosphorylation
Initiation of sperm motility
Head
Mitochondria
Flagellum
C: Sea urchins
Peptides e.g. speract, resact
ANP receptor
Guanylyl cyclase active
cGMP↑
K+ efflux, membrane hyperpolarization
Na+/H+ exchange
[pH]i↑
Protein phosphorylation
Phosphoryl creatine shuttle
Adenylyl cyclase active
Dynein ATPase active
cAMP↑
ATP
Ca2+ channel
Microtubule sliding
[Ca2+]i↑
Initiation & activation of sperm motility and respiration
Chemotaxis (resact)
Potentiation of acrosome reaction (speract)
B: Mammals
Protease activity
HCO3- influx
Adenylyl cyclase active
[Ca2+]i↑, [pH]i↑
cAMP↑
Ca2+-calmodulin
56kDa axokinin phosphorylation
Phosphatase active
Phosphorylation and dephosphorylation of outer arm dynein
Regulation of ATPase activity and microtubule sliding
Initiation, activation of sperm motility
D: Ciona
SAAF
T-type Ca2+ channel
[Ca2+]i↑
cAMP↑
Phosphorylation
Initiation and activation of sperm motility
Chemotaxis

$[pH]_i$ recover on subsequent addition of zinc, suggesting that the increase of $[pH]_i$ resulting from exposure of sperm to zinc in sea water, is a possible cause of initiation of motility of sea urchin sperm. In contrast, the respiration and motility of starfish sperm are increased by chelators which reduced the level of zinc [102, 184]. Starfish spermatozoa initially contain zinc, but release it into the surrounding seawater, upon dilution and motility activation [100], suggesting that this release of zinc causes initiation of sperm motility. The concentrations of heavy metals in sea urchin sperm have been measured [105] and the effect of zinc on microtubule proteins [106], for example conformational changes of flagellar protein [103] and tubulin assembly [67], and sheet polymer formation [81, 84] have been studied.

In the horseshoe crab, *Limulus polyphemus*, spermatozoa are immotile upon dilution in seawater and become motile on release of a peptide from the eggs [27, 29]. Mobilization of intracellular Ca^{2+} and an increase in the cAMP level have been correlated with initiation of motility by a crude egg extract of the horseshoe crab as well as the starfish [180]. Trace metals and divalent cations are also involved in the regulating sperm motility of the horseshoe crab [28, 31]. Zinc is absorbed by the sperm from the surrounding medium and inhibits their motility, whereas removal of zinc from the sperm by treatment with a chelating agent causes initiation of sperm motility [31].

There are several reports that the semen or spermatozoa of mammals such as dogs, humans and rats, contain a high concentration of zinc (see [89, 148]). Zinc is closely associated with sulfhydryl groups and disulfide linkages and is concentrated in the sperm tail [64]. Sperm contain heavy metals [48, 90, 148], and their removal from the sperm with a chelating agent [148] enhances or inhibits sperm motility [82, 91, 145, 147] as well as in marine invertebrates (see above). Thus zinc may be necessary for regulation of sperm motility in various phyla, from invertebrates to vertebrates.

V CONCLUSIONS

Drastic change in the motility state occurs in the life history of spermatozoa. During transit of sperm from the testis to the epididymis or vas deferens, spermatozoa generally acquire the potential for motility [117, 179], although there are some exceptions [10], and cAMP, Ca^{2+} and pH are known to be important regulators of the process [68, 118, 119]. The subject of "acquisition of sperm motility" was not included in this review.

Even though spermatozoa have capacity to move, their motility is more or less suppressed in the epididymis or vas deferens by factors present in the fluid part of the semen. Assuming that the motility observed in the undiluted sperm samples represents that within a male reproductive organ, such as the epididymis or sperm duct, spermatozoa are completely quiescent in sea urchins [56] and teleosts [107], whereas the spermatozoa in some species, especially some mammals, are motile to a certain degree [123]. On spawning into an aquatic environment in species with external fertilization or at ejaculation into the female reproductive tract after mixing with the seminal plasma in species with internal fertilization, sperm change from an immotile or slightly motile state to a fully motile state, named "initiation of sperm motility". Then their motility is activated, "activation of sperm motility", and they are attracted toward eggs, "sperm chemotaxis".

The cell signaling systems underlying these changes in motility are various. However, the external signals for initiation of sperm motility on spawning and ejaculation are basically changes in the environment of inorganic components such as CO_2, ions including Ca^{2+} and zinc or in non-chemical stimuli, such as osmolality and temperature. Activation and chemotaxis, in contrast, are caused by exposure to organic compounds, mainly peptides or proteins released from the female. The spermatozoa of *Ciona* and the herring, remain quiescent or only slight motile for a while after spawning. In such cases, initiation and activation of sperm motility may be induced simultaneously on stimulation by an organic substance from the female. In both species as well as in *Arbacia punctulata*, one substance, such as SAAF, HSAPs or resact may have the ability to induce plural phenomena. For instance, SAAF from *Ciona* eggs causes initiation, activation, and chemotaxis of sperm spawned in seawater.

Internal cascade systems for sperm motility are triggered by several factors, mainly second messengers, such as cyclic nucleotides and Ca^{2+}, and seem to converge upon the phosphorylation and dephosphorylation of proteins through several kinds of protein kinases and phosphatases which would be related to axonemal movement. The phosphorylation of protein for initiation of motility was found to occur at two different sites. Cyclic AMP-dependent phosphorylation of motility initiating phosphoprotein (MIPP) in salmonid fishes is suggested to occur in the basal portion of the flagellum, possibly the centriolar region, whereas both cAMP-dependent phosphorylation of axokinin and calmodulin-dependent dephosphorylation of dynein ATPase appear to occur all along the whole length of the axoneme and to modulate sliding of microtubules in mammalian spermatozoa (Fig. 7). The molecular mechanism indispensable for the control of flagellar motility has generally been thought to be distributed along the axoneme. In a recent review, however Brokaw discusses the special function of the basal end of the flagellum [21]. In initiation of chicken sperm motility, the

Fig. 7. Proposed cell signaling mechanisms underlying the initiation and activation of sperm motility, and chemotaxis in the sea urchin, *Ciona*, salmonid fishes and mammals. A: from Morisawa M. (this review). B: from Okamura *et al.*(shaded line, [133]); Tash JS (black line, [172]). C: from Domino SE and Garbers DL(black line, [45]); Cook SP and Babcock DF (shaded line, [36]); Tombes RM and Shapiro BM (dotted line, [178]). D: From Yoshida *et al.* [199]

external temperature directly affects the axoneme and Ca^{2+} controlling the functions of other systems in soluble components, suggesting the presence of two systems for motility initiation in different portions of the sperm cell.

The facts that chemoattractants have been shown to modify the flagellar asymmetry in a Ca^{2+}-dependent manner in siphonophores [39] and in *Ciona* [199], suggest a role of the attractants in regulation of $[Ca^{2+}]_i$. However, the target of Ca^{2+} has not been identified. For initiation and activation of sperm motility, the main cell signaling pathway is cyclic nucleotide-dependent, whereas the chemotactic behavior of sperm may be modulated by Ca^{2+} rather than cAMP.

Efforts to identify the molecular mechanisms of cell signaling for the regulation of sperm motility should contribute to our understanding of general mechanisms of cell signaling in cells.

ACKNOWLEDGMENTS

I thank my colleagues Drs. Hiroshi Hayashi, Sachiko Morisawa, Makoto Okuno, Katsumi Ishida, Hideki Ohtake, Ken-ichi Manaka, Satomi Tanimoto and Zong Xuan Jin, and the graduate students of Misaki Marine Biological Station, Syoji Oda, Hiroyuki Takai and Manabu Yoshida whose findings have been discussed in this review. I am also indebted to Dr. Hideo Mohri, Professor Emeritus of the University of Tokyo for his encouragement. This work was supported by Grants-in-Aid from the Ministry of Education, Science and Culture of Japan.

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